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**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Application Number: 10/526,026
Filing Date: August 11, 2005
Appellant(s): YAMAOKA ET AL.

Douglas P. Mueller
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 03/31/2010 appealing from the Office action mailed 09/01/2009

(1) Real Party in Interest

A statement identifying the real party interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

A statement that there are no related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief

(3) Status of Claims

The statement of the status of the claims contained in the brief is correct. The following is a list of claims that are rejected and pending in the application:

Claims 1, 6-9, 11-14, 24-26 are pending and rejected.

(4) Status of Amendments After Final

No amendments after final rejection have been filed.

(5) Summary of Claimed Subject Matter

The examiner has no comment on the summary of claimed subject matter contained in the brief.

(6) Grounds of Rejection to be Reviewed on Appeal

The examiner has no comment on the appellant's statement of the grounds of rejection to be reviewed on appeal. Every ground of rejection set forth in the Office action from which the appeal is taken (as modified by any advisory actions) is being maintained by the examiner except for the grounds of rejection (if any) listed under the subheading "WITHDRAWN REJECTIONS." New grounds of rejection (if any) are provided under the subheading "NEW GROUNDS OF REJECTION."

Art Unit: 1652

(7) Claims Appendix

The examiner has no comment on the copy of the appealed claims contained in the Appendix to the appellant's brief.

(8) Evidence Relied Upon

WO 02/36779 SODE 5-2002

2004/0023330 SODE 2-2004

Shimomura, Y, Nishikimi, M. and Ozawa, T. "Purification of the Iron-sulfur Protein, Ubiquinone-Binding Protein, and Cytochrome c₁ from a Single Source of Mitochondrial Complex III", Analytical Biochemistry, vol. 153, (1986), pages 126-131

The 1999 AMERSHAM Catalog, pages 520, 523 and 527.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1, 6-9, 11-14 and 24-26 have been rejected under 35 U.S.C. 103(b) by Sode (WO 02/36779, English translation in US 2004/0023330) in view of Shimomura et al (Analytical Biochemistry 1986 vol 153, pp 126-131) and further in view of the 1999 AMERSHAM catalog (pages 520, 523 and 527).

Art Unit: 1652

Claims 1, 6-9, 11-14 and 24-26 are directed in part to the purification of a *Burkholderia cepacia* KSI strain glucose dehydrogenase (GDH) protein comprising α , β , and γ subunits using liquid chromatography, wherein an ion exchange resin comprising quaternary ammonium group and an eluent containing cholate are employed.

Sode teaches a GDH protein produced from the microorganism *Burkholderia cepacia* KSI strain (FERM BP-7306, see claims 7 and 30; paragraph 0087) comprising α , β , and γ subunits and teaches expression of said GDH protein in *E. coli* (paragraphs 0137-0138) as well as the purification of said GDH protein by ion-exchange chromatography using a DEAE-Toyopearl column and a buffer at pH 8.0 (a non-acidic pH; paragraph 0175). Sode also teaches that said GDH protein comprises a cytochrome c (paragraphs 0033-0039) type protein associated with the cell membrane (purified from the membrane fraction of the cell, paragraphs 0173-0174). However, Sode does not teach the elution of said protein from the DEAE-Toyopearl column with a sodium cholate buffer or liquid chromatography using a resin containing a quaternary ammonium group.

Shimomura et al. teach the purification of subunits of cytochrome bc (also called Complex III, page 126, Abstract and first line of first paragraph, left column) using liquid chromatography, wherein a phenyl-Sepharose resin and an eluent buffer comprising cholate was used. The eluent was applied at a constant gradient (page 127, left column, Resolution of Complex III and purification of subunits). Shimomura et al. teach that Complex III was applied to a phenyl-Sepharose CL-4B column and that (1) iron-sulfur protein (component of Complex III) was eluted with a buffer containing Tris-HCl and 1% deoxycholate at pH 8.0, (2) subunit VI (Component of complex III) was eluted with a buffer containing guanidine hydrochloride dissolved in buffer A, wherein buffer A contains 25 mM Tris-Cl, 1% cholate, 1 mM DTT and 20% glycerol, (3) core proteins I and II (subunits of Complex III) were eluted with a buffer containing guanidine hydrochloride dissolved in buffer A, and (4) cytochrome c_1 (subunit of Complex III)

Art Unit: 1652

was eluted with buffer A containing 1% C₁₂E₈. Shimomura et al. teach that the remaining subunits were eluted with buffer A containing 2% SDS (page 127, right column, first three lines).

It is well known in the art and evidenced by the teachings of Shimomura et al. discussed above, that membrane-associated proteins are hydrophobic in nature and detergent type molecules are used to solubilize and purify membrane-associated proteins. For example, Shimomura et al. teach detergent-exchange chromatography to separate subunits of Complex III (Abstract). Cholate behaves as a detergent and is used in eluent buffers for the purification of different membrane-associated proteins because it facilitates hydrophobic-interaction. Protein is adsorbed in the column matrix and cholate aids in eluting the protein from the column due to the hydrophobicity of cholate.

Q-Sepharose is an ion-exchange column resin comprising a quaternary ammonium moiety and commercially available from AMERSHAM (see the 1999 AMERSHAM Catalog, page 523). The advantages of using Q-Sepharose as an anion exchanger in protein purification are well documented, such as high flow, high capacity, reproducibility, industrial scale application and commercial availability (see the 1999 AMERSHAM catalog, pages 520, 523). Q-Sepharose has several advantages over DEAE columns, such as wide pH ranges, high resolution, and high capacity for the protein to be separated in high salt concentration.

As such, it would have been obvious to one of ordinary skill in the art to purify a GDH protein comprising α , β , and γ subunits from the microorganism *Burkholderia cepacia* KSI strain or produced by an *E. coli* transformant (as taught by Sode) using a method of purification that requires liquid chromatography, wherein a Q-Sepharose column is used, wherein a buffer containing cholate is used as the eluent, wherein a constant gradient is applied, and wherein the concentration of cholate in the eluent is 0.5-2.5 wt%. A person of ordinary skill in the art is motivated to modify the purification protocol of Sode and use Q-Sepharose as the ion exchange resin and an eluent buffer containing cholate because Sode teaches that the GDH protein is a membrane-associated protein, Shimomura et al. teach the purification of

Art Unit: 1652

membrane-associated proteins with ion-exchange chromatography using a cholate-containing eluent buffer, and the AMERSHAM catalog teaches the many advantages of using Q-Sepharose as an ion exchange resin over other ion exchange resins. One of ordinary skill in the art has a reasonable expectation of success at purifying the GDH protein of Sode by modifying the purification protocol of Sode and use Q-Sepharose and a cholate-containing eluent buffer in view of the successful purification of membrane-associated proteins, including a cytochrome c_1 protein, using ion-exchange chromatography and a cholate-containing eluent buffer as taught by Shimomura et al., and the fact that Q-Sepharose is a well known ion-exchange resin which has several advantages over other ion-exchange resins. Therefore, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made.

(10) Response to Argument

On pages 4-10 of the Brief, Appellants argue that the Examiner has not shown a *prima facie* case of obviousness because (A) there is no suggestion or motivation to modify or combine the teachings of Shimomura et al. with the teachings of Sode and AMERSHAM, and (B) there is no reasonable expectation of success based on Shimomura et al., Sode and AMERSHAM that would lead one of skill in the art to Appellant's invention. The Examiner will address each argument in the order presented by Appellant in the Brief.

A. There is no suggestion or motivation to modify or combine the teachings of Shimomura et al. with the teachings of Sode and Amersham

On page 5 of the Brief, Appellant argues that in order for references to be combined in an obviousness rejection, there must be a source of motivation to modify the teachings of one reference using the teachings of other references. On pages 6-8 of the brief, Appellant argues that while Shimomura et al. teach the purification of Complex III by serially eluting iron-sulfur protein, cytochrome c_1 , and ubiquinone-binding protein by changing elution buffers, as well as the use of an eluent containing

Art Unit: 1652

cholate, the reference fails to disclose or suggest a glucose dehydrogenase, or a resin containing a quaternary ammonium group. With regard to Sode, Appellant argues that Sode fails to disclose or suggest elution with cholate or an ion exchange resin having a quaternary ammonium group. Appellant acknowledges the teachings of the 1999 AMERSHAM catalog but submits that there is no motivation to combine the cited references. Appellant argues that Shimomura et al. teach deoxycholate with sodium chloride, and uses other eluents containing guanidine hydrochloride and SDS. Appellant further argues that simply because an ion exchange resin containing a quaternary ammonium group was available in the AMERSHAM catalog, that does not rise to an inference that such packing agent would be desirable for purifying a GDH protein or that a cholate-containing buffer should be used as an eluent.

Appellant's arguments have been fully considered but found not persuasive. As indicated previously, Shimomura et al. teach the purification of the subunits of Complex III using a cholate-containing elution buffer using an ion exchange resin. It is noted that Shimomura et al. teach that cholate was present in all the elution buffers used to purify subunit VI, core proteins I and II, and cytochrome c_1 , whereas deoxycholate was present in the elution buffer used to purify iron-sulfate protein. The elution buffers used to purify subunit VI, core proteins I and II, and cytochrome c_1 all contained cholate as all of these buffers contained buffer A, which comprises 1% cholate. See page 127, left column. Therefore, while it is acknowledged that the elution buffers used to purify subunit VI and core proteins I and II contained guanidine hydrochloride, all these buffers also contained cholate by virtue of having been made with buffer A. Also, as indicated previously, Shimomura et al. teach that all the remaining subunits of Complex III were purified by eluting them with a buffer containing buffer A and SDS, thus also being eluted with a buffer containing cholate (page 127, right column, first three lines).

As known in the art as evidenced by Shimomura et al., and also admitted by Appellant in the response of 12/13/2006, cholate-containing buffers are used in ion-exchange chromatography for the purification of membrane-associated proteins. See, for example, Appellant's statement on page 7 of the

Art Unit: 1652

response filed on 12/13/2006 (second full paragraph), where Appellant states “O’Riordan discloses purification of membrane-associated proteins using, for example, ion-exchange chromatography using eluent containing cholate”. Cholate behaves as detergent and is used in elution buffers for the purification of different membrane-associated proteins because membrane-associated proteins are hydrophobic and cholate facilitates hydrophobic interaction. Protein is adsorbed in the column matrix and cholate is used to elute the membrane-associated protein as it contains a hydrophobic component. Therefore, the motivation to use cholate-containing buffers is clearly found in the teachings of Shimomura et al. as well as the knowledge of one of ordinary skill in the art regarding the use of cholate in ion exchange chromatography since the GDH protein of Sode is a membrane-associated protein comprising a cytochrome c just like the protein purified by Shimomura et al. (Complex III).

With regard to Q-Sepharose, it is well known in the art as evidenced by the 1999 AMERSHAM catalog, that Q-Sepharose is a good choice for an ion-exchange resin. As previously indicated, Q-Sepharose has several advantages over other ion-exchange resins such as wide pH ranges, high resolution, and high capacity for the protein to be separated in high salt concentrations. As such, the motivation to use Q-Sepharose is found in the fact that this resin has several properties which makes it highly desirable over other ion exchange resins. It should also be noted that there is a limited number of ion exchange resins and detergents used in biology known in the art. Therefore, the instant case is not one where there is an unlimited number of possibilities but rather one where there is a finite number of predictable alternatives. The claimed invention is simply the replacement of the ion exchange resin used by Sode (DEAE-Toyopearl column) with an ion exchange resin well known in the art for having highly desirable properties (Q-Sepharose) and the replacement of the elution buffer of Sode with a buffer containing a detergent-like compound (cholate) well known in the art for purification of membrane-associated proteins, such as the GDH protein of Sode. Therefore, contrary to Appellant’s assertions, one of skill in the art would have been highly motivated to combine the teachings of Sode, Shimomura et al. and the

Art Unit: 1652

1999 AMERSHAM catalog and use Q-Sepharose as the ion exchange resin and a cholate-containing buffer as the elution buffer for the benefit of purifying a GDH protein, which is a membrane-associated protein.

B. There is no reasonable expectation of success based on Shimomura et al., Sode and Amersham that would lead one of skill in the art to appellant's invention.

On pages 8-9 of the Brief, Appellant argues that there is nothing in the prior art that would lead one of skill in the art to predict the excellent effects demonstrated by Appellant's claimed method. Appellant refers to the results discussed in the specification as to the higher final specific activity obtained with an ion exchange resin containing a quaternary ammonium group and elution with a cholate-containing buffer. Appellant argues that the results reported by Appellant would have been unexpected to one of ordinary skill in the art.

Appellant's arguments have been fully considered but not found persuasive. There is nothing surprising or unexpected in purifying a GDH protein comprising α , β , and γ subunits using an ion exchange column with a resin comprising quaternary ammonium group and a cholate-containing buffer as an eluent. Many proteins and enzymes have been purified by liquid chromatography using resins comprising quaternary ammonium groups, such as the Q-Sepharose resin described in the 1999 AMERSHAM Catalog, using a variety of eluents. Similarly, the use of cholate-containing elution buffers in combination with ion exchange resins to purify membrane-containing proteins is well known in the art, as evidenced by Shimomura et al. and admitted by Appellant in the response of 12/13/2006. Improved purity of the GDH protein using a purification protocol like the one taught by Shimomura et al. with Q-Sepharose is expected because the use of detergents such as cholate facilitates solubilization of membrane-associated proteins with minimum denaturation resulting in greater activity of the desired protein, and Q-Sepharose is a well known resin which has several advantages over other ion exchange resins.

In view of the teachings of Shimomura et al., the 1999 AMERSHAM catalog, Sode and the knowledge of one of ordinary skill in the art regarding ion exchange resins and the use of detergents in elution buffers for purification of membrane-associated proteins, one of skill in the art would have a reasonable expectation of purifying another membrane-associated protein comprising a cytochrome c subunit like the protein purified by Shimomura et al. using Q-Sepharose and a cholate-containing elution buffer. While there is no absolute certainty that the GDH protein will be purified by the method of Shimomura et al., Sode and the 1999 AMERSHAM catalog, all that is required to show a *prima facie* case of obviousness is a reasonable expectation of success. With regard to arguments of unexpected results and the higher final specific activity obtained when an ion exchange resin containing a quaternary ammonium group and a cholate-containing buffer were used, it is noted that the claims do not require any particular level of purification for the GDH protein. There are no limitations in the claims regarding the level of purity of the GDH protein. Therefore, even if the argument is made that the high purity observed was unexpected, that argument is deemed irrelevant because the claims do not require a specific level of purification. The claims merely require purification of the GDH protein. Since there is a reasonable expectation of success at purifying the GDH protein using the method of Shimomura et al., Sode and the 1999 AMERSHAM catalog, and there is motivation to combine the teachings of the cited references for the reasons extensively discussed above, it is believed that a *prima facie* case of obviousness has been established. The Examiner has provided the rationale to support a conclusion that the claims would have been obvious in that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination yielded nothing more than predictable results to one of ordinary skill in the art; KSR, 550 U.S.398,419 (2006); Sakraida v. AG Pro, Inc., 425 U.S. 273, 282, 189 USPQ 449, 453 (1976); Anderson 's-Black Rock, Inc. v. Pavement Salvage Co., 396 U.S. 57, 62-63, 163 USPQ 673, 675 (1969);

Art Unit: 1652

Great Atlantic & P. Tea Co. v. Supermarket Equipment Corp., 340 U.S. 147, 152, 87 USPQ 303, 306 (1950).

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

Mohammad Younus Meah Ph.D.

Art Unit 1652

Conferees:

/Robert B Mondesi/
Supervisory Patent Examiner, Art Unit 1652

/Terry A. McKelvey/
Supervisory Patent Examiner, Art Unit 1655

/Delia M. Ramirez/
Primary Examiner, Art Unit 1652